

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

LISTING OF CLAIMS

1. (Currently amended) A recombinant genetic construct ~~which is suitable for an insertion/deletion or an inversion for at least one target nucleotide sequence, said genetic construct comprising:~~

a promoter/activator sequence disposed upstream of a first nucleotide sequence encoding a first ~~toxice-moleeule~~ poison protein and disposed upstream of a second nucleotide sequence encoding a second ~~toxice-moleeule~~ poison protein different from said first ~~toxice-moleeule~~ poison protein, or

a first promoter/activator sequence disposed upstream of a first nucleotide sequence encoding a first ~~toxice-moleeule~~ poison protein, wherein said first nucleotide sequence encoding a first poison protein is flanked by recombination sites or restriction sites, and ~~disposed in an opposite direction of said first nucleotide sequence~~, and a second promoter/activator sequence disposed upstream of a second nucleotide sequence encoding an antidote to a second ~~toxice-moleeule~~ poison protein different from said first ~~toxice-moleeule~~ poison protein, or

a promoter/activator sequence disposed upstream of a first nucleotide sequence encoding a first ~~toxice-moleeule~~ poison protein and disposed upstream of a second nucleotide sequence encoding an antidote to a second ~~toxice-moleeule~~ poison protein different from said first ~~toxice-moleeule~~ poison protein, wherein said first nucleotide sequence encoding a first poison protein is flanked by recombination sites or restriction sites,

wherein the poison/antidote proteins are selected from the group consisting of CcdB/CcdA, Kid/Kis, Doc/Phd, RelE/RelB, PasA/PasB/PasC, MazE/MazF and ParE/ParD.

2. (Currently amended) The genetic construct according to claim 1 ~~suitable for the inversion of at least one target nucleotide sequence and comprised of comprising:~~

a first promoter/activator sequence disposed upstream of a first nucleotide sequence encoding a ~~toxic-molecule~~ poison protein and a second nucleotide sequence encoding an antidote to a second ~~toxic-molecule~~ poison protein different from said first ~~toxic-molecule~~ poison protein wherein the second nucleotide sequence is disposed in an opposite direction to the reading orientation of the first promoter/activator sequence.

3. (Currently amended) The genetic construct according to claim 2, wherein a third nucleotide sequence encoding an antidote to the first ~~toxic-molecule~~ poison protein is under the control of a second promoter/activator sequence.

4. (Currently amended) The genetic construct according to claim 1, wherein each nucleotide sequence encoding a ~~toxic-molecule~~ poison protein or an antidote to a ~~toxic-molecule~~ poison protein is a nucleic acid sequence which encodes a fusion protein active as a ~~toxic-molecule~~ poison protein or as an antidote to said ~~toxic-molecule~~ poison protein, said nucleic acid sequence which encodes said fusion protein comprising several unique cloning sites and a nucleotide sequence encoding a ~~molecule-toxic-to-a-cell~~ poison protein or an antidote to said ~~toxic-molecule~~ poison protein.

5. (Currently amended) The genetic construct according to claim 1, which further comprises recombination sites disposed upstream and downstream the nucleotide sequence(s) encoding the first and the second ~~toxics-molecules~~ poison proteins ~~and/or the nucleotide sequence(s) encoding an antidote to a toxic molecule.~~

6. (Canceled)

7. (Canceled)

8. (Previously presented) A cloning vector comprising the genetic construct according to claim 1.

9. (Previously presented) The cloning vector according to claim 8 further comprising an origin of replication and a selectable marker.

10. (Previously presented) A cell transformed by the genetic construct according to claim 1.

11. (Previously presented) The cell according to claim 10 which is selected from the group consisting of prokaryote cells, plant cells, animal cells and fungi cells.

12. (Currently amended) A cloning and selection kit comprising ~~an element selected from the group consisting of one or more nucleic acid constructs according to claim 1,~~ one or more vectors comprising at least one genetic construct according to claim 1 and cells to be transformed by said construct or vector, wherein said cells are either resistant or sensitive to one or more of the ~~toxie-molecule(s)~~ poison proteins, or wherein said cells are expressing one or more of said ~~toxie-molecule(s)~~ poison proteins or ~~antidote(s)~~ antidotes to the said ~~toxie-molecule(s)~~ one or more poison proteins.

13. (Withdrawn) A method ~~for an insertion and/or an inversion of a target nucleotide sequence into a nucleic acid construct~~ said method comprising the following steps:

providing a nucleic acid construct according to claim 1 and obtaining the insertion of the target nucleotide sequence into the nucleic acid construct by inactivation of a nucleotide sequence encoding a ~~toxie-molecule~~ poison protein and,

selecting the nucleic acid construct having integrated the target nucleotide sequence in a cell which is sensitive to said ~~toxie-molecule~~ poison protein.

14. (Withdrawn) The method according to claim 13 further comprising:

selecting the target nucleotide sequence from a genome database through analysis of a corresponding genomic sequence by identification of exon-intron-structure and comparison with expression genetic databases,

providing primer sequences suitable for a genetic amplification and cloning of said target genetic sequence,

selecting elements of the nucleic acid construct presented in databases as well as cells to be transformed by the nucleic acid construct, and

providing a design of the nucleic acid construct suitable for an integration of the target nucleotide sequence.

15. (Withdrawn) The method according to claim 13 which further comprises the step of replacing the target nucleotide sequence by elements that have been deleted following an insertion of said target nucleotide sequence or by the integration of a target nucleotide sequence having an inverted reading orientation.

16. (Withdrawn) The method according to claim 15, wherein said integration of the target nucleotide sequence, replacement or inversion of the target nucleotide sequence is a step selected from the group consisting of restriction/ligation, site specific recombination, TOPO cloning and homologous recombination steps.

17. (Withdrawn) The method according to claim 16, which comprises the step of insertion/deletion and/or reversion of several target nucleotide sequences into multiple nucleic acid construct(s) and the step of selecting simultaneously a construct having integrated, deleted or inverted said target nucleotide sequences.

18. (Withdrawn) The method according to claim 17, wherein the step of selecting simultaneously a construct having integrated, deleted or inverted the target nucleotide sequences is made in a single cell or in a single reaction tube.

19. (Withdrawn) A computer program comprising program codes means for performing the steps according to claim 13.

20. (Withdrawn) A computer program product comprising the program codes means on a computer readable medium for performing the steps of the method according to claim 13 when said program is run on a computer.

21. (Withdrawn) A robot connected to a database of a computer and which comprises an element selected from the group consisting of the genetic construct according to claim 1, a vector comprising at least one genetic construct according to claim 1, a cell transformed by the genetic construct according to claim 1, and a kit comprising at least one genetic construct according to claim 1.

22. (Currently amended) The genetic construct of claim 2, wherein said second nucleotide sequence encoding said antidote to said second ~~toxic molecule~~ poison protein is under the control of the second promoter/activator sequence.

23. (Previously presented) The vector of claim 9, wherein the selectable marker is an antibiotic resistance selectable marker.

24. (Previously presented) The cell of claim 11, wherein said animal cells are human cells.

25. (Previously presented) The cell of claim 11, wherein said fungi cells are yeast cells.

26. (Withdrawn) A robot connected to a database of a computer and which comprises a computer program that includes a means for performing an insertion and/or an inversion of a target nucleotide sequence into a nucleic acid construct, said means comprising the following steps:

defining a nucleic acid construct comprising a promoter/activator sequence disposed upstream of a first nucleotide sequence, encoding a first toxic molecule and a second nucleotide sequence encoding a second toxic molecule different from said first toxic molecule, or a first promoter/activator sequence disposed upstream of the first nucleotide sequence encoding a first toxic molecule and disposed in an opposite direction of said first nucleotide sequence and a second promoter/activator sequence disposed upstream of a second nucleotide sequence encoding an antidote to a second toxic molecule different from said first toxic molecule, or a promoter/activator sequence disposed upstream of a first nucleotide sequence encoding the first toxic molecule and a second nucleotide sequence encoding an antidote to the second toxic molecule different from said first toxic molecule;

obtaining the insertion of the target nucleotide sequence into the nucleic acid construct by inactivation of a nucleotide sequence encoding a toxic molecule, and

selecting the nucleic acid construct having integrated the target nucleotide sequence in a cell which is sensitive to said toxic molecule.

27. (New) The genetic construct according to claim 1 lacking a foreign target nucleotide sequence.

28. (New) The genetic construct according to claim 1, wherein said construct comprises a promoter/activator sequence disposed upstream of a first nucleotide sequence encoding a first poison protein and disposed upstream of a second nucleotide sequence encoding a second poison protein different from said first poison protein.

29. (New) The genetic construct according to claim 28 lacking a foreign target nucleotide sequence.

30. (New) The genetic construct according to claim 1, wherein said construct comprises a first promoter/activator sequence disposed upstream of a first nucleotide sequence encoding a first poison protein, wherein said first nucleotide sequence encoding a first poison

protein is flanked by recombination sites or restriction sites, and a second promoter/activator sequence disposed upstream of a second nucleotide sequence encoding an antidote to a second poison protein different from said first poison protein.

31. (New) The genetic construct according to claim 30, wherein said first nucleotide sequence encoding a first poison protein is flanked by recombination sites.

32. (New) The genetic construct according to claim 31, wherein said first promoter/activator sequence and said first nucleotide sequence encoding a first poison protein are together flanked by recombination sites.

33. (New) The genetic construct according to claim 31, wherein an antibiotic resistance marker is not present between said recombination sites.

34. (New) The genetic construct according to claim 30, wherein said first promoter/activator sequence is disposed in an opposite direction of said first nucleotide sequence.

35. (New) The genetic construct according to claim 30 lacking a foreign target nucleotide sequence.

36. (New) The genetic construct according to claim 1, wherein said construct comprises a promoter/activator sequence disposed upstream of a first nucleotide sequence encoding a first poison protein and disposed upstream of a second nucleotide sequence encoding an antidote to a second poison protein different from said first poison protein, wherein said first nucleotide sequence encoding a first poison protein is flanked by recombination sites or restriction sites.

37. (New) The genetic construct according to claim 36, wherein said first nucleotide sequence encoding a first poison protein is flanked by recombination sites.

38. (New) The genetic construct according to claim 37, promoter/activator sequence and said first nucleotide sequence encoding a first poison protein are together flanked by recombination sites.

39. (New) The genetic construct according to claim 37, wherein an antibiotic resistance marker is not present between said recombination sites.

40. (New) The genetic construct according to claim 36 lacking a foreign target nucleotide sequence.